#knowledge/internet

How the Coronavirus infects our cells

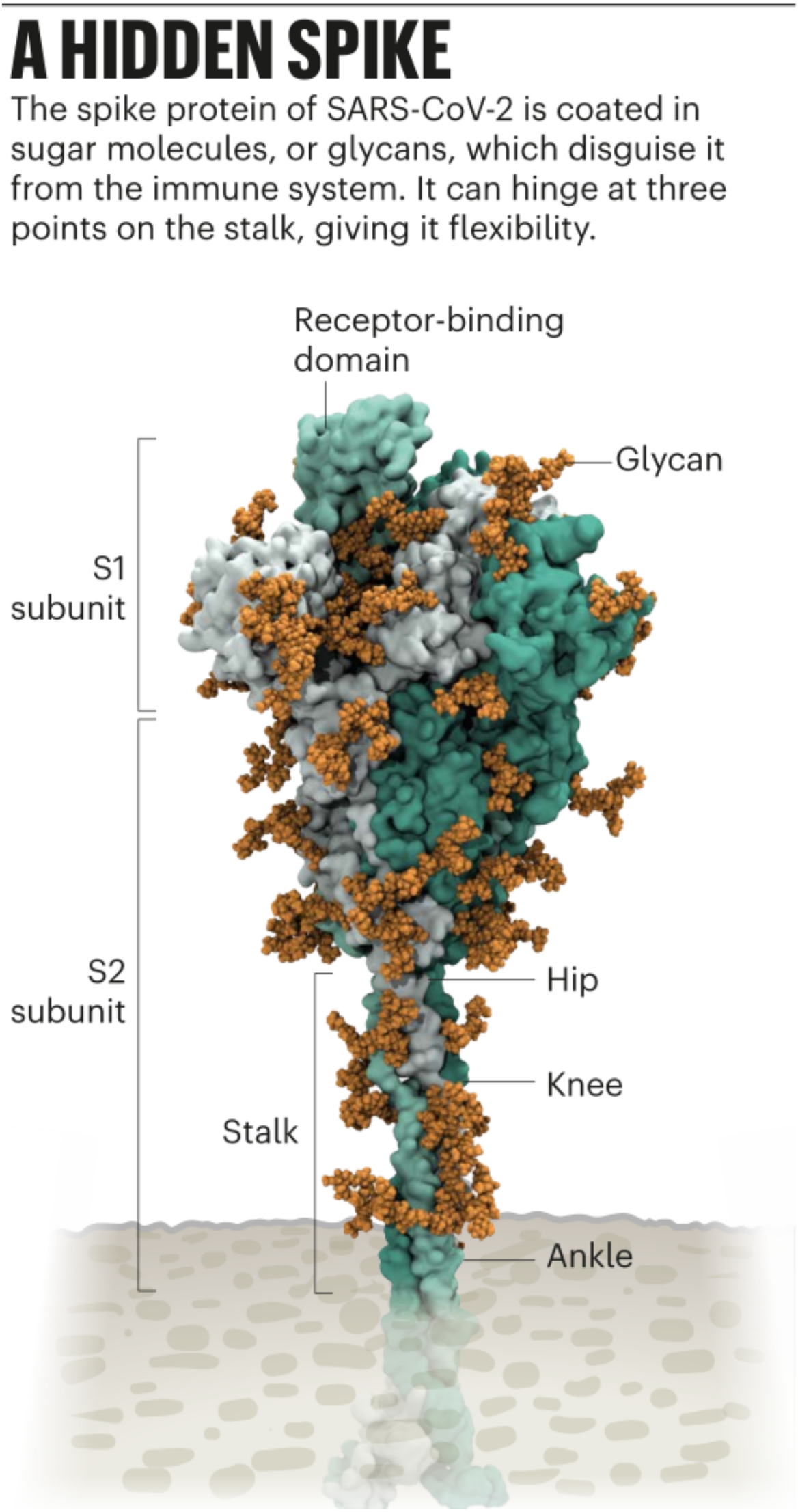
Scientists are unpicking SARS-CoV-2's life cycle. By **Megan Scudellari**

Introduction

- Many viruses have glycans covering their outer proteins, camouflaging them from the human immune system.
- The spike protein has an RBD (receptor binding domain) that can hinge at three points on the spike, giving it flexibility.
- The RBD is hidden by two glycans. It's possible that snipping out those two sugars could reduce the virus's infectivity, says Amaro, although researchers don't yet have a way to do this.

Barbed and Ready

- Each SARS-CoV-2 virion (virus particle) has an outer surface peppered with 24–40 haphazardly arranged spike proteins that are its key to fusing with human cells.
- For other types of virus, such as influenza, external fusion proteins are relatively rigid. SARS-CoV-2 spikes, however, are wildly flexible and hinge at three points.
- The RBDs of SARS-CoV-2 spike proteins attach to a familiar protein called the ACE2 receptor, which adorns the outside of most human throat and lung cells.



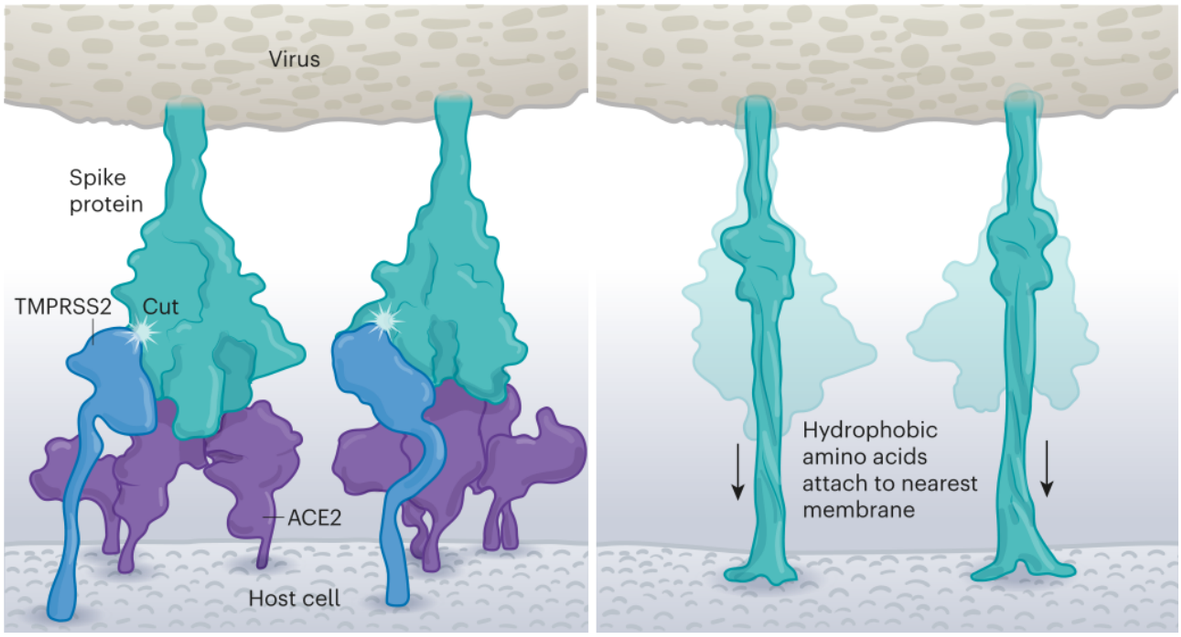
Worrying variants of SARS-CoV-2 tend to have mutations in the S1 subunit of the spike protein, which hosts the RBDs and is responsible for binding to

the ACE2 receptor.

Restricted Entry

- Once the viral spikes bind to ACE2, other proteins on the host cell's surface initiate a process that leads to the merging of viral and cell membranes.
- There are two ways of entry for SARS-CoV, using the TMPRSS-2 or cathepsin L enzymes. SARS-CoV used the latter but it was easy for antiviral proteins to trap them. SARS-CoV-2 uses TMPRSS-2, an enzyme found in high amounts on the outside of respiratory cells.
- First, TMPRSS2 cuts a site on the spike's S2 subunit. That cut exposes a run of hydro-phobic amino acids that rapidly buries itself inthe closest membrane — that of the host cell.
- Next, the extended spike folds back onto itself, like a zipper, forcing the viral and cell membranes to fuse.
- The virus then ejects its genome directly into the cell. By invading in this spring-loaded manner, SARS-CoV-2 infects faster than SARS-CoV and avoids being trapped in endosomes.
- **VIRAL ENTRY UP CLOSE**

Virus and host-cell membranes fuse after the TMPRSS2 enzyme cuts a SARS-CoV-2 spike protein. This exposes hydrophobic amino acids in the spike that rapidly embed themselves into the nearest membrane — that of the host cell.



- This is why the malaria drug chloroquine didn't work. Chloroquine is good at disrupting the cathepsin using viruses.
- The discovery also points to protease inhibitors as a promising therapeutic option to prevent a virus from using TMPRSS2, cathepsin L or other proteases to enter host cells.
- One TMPRSS2 inhibitor, camostat mesylate, which is approved in Japan to treat pancreatitis, blocked viral entry into lung cells, but the drug did not improve patients' outcomes in an initial clinical trial.

Deadly Competition

- The next steps of infection are murkier. There's more uncertainty, and competing hypotheses.
- After the virus shoots its RNA genome into the cell, ribosomes in the cytoplasm translate two sections of viral RNA into long strings of amino acids, which are then snipped into 16 proteins, including many involved in RNA synthesis. Later, more RNAs are generated that code for a total of 26 known viral proteins, including structural ones used to make new virus particles, such as the spike, and other accessory proteins. In this way, the virus begins churning out copies of its own messenger RNA. But it needs the cell's machinery to translate thise mRNA into proteins.
- Viral protein Nsp1, one of the first proteins translated when the virus arrives, recruits host proteins to systematically chop up all cellular mRNAs that don't have a viral tag.
- Second, infection reduces overall protein translation in the cell by 70%. Nsp1 is again the main culprit, this time physically blocking the entry channel of ribosomes so mRNA can't get inside. The little translation capacity that remains is dedicated to viral RNAs.
- The virus prevents cellular mRNA from getting out of the nucleus, including instructions for proteins meant to alert the immune system to infection.
- Because gene transcripts can't get out of the nucleus, the infected cells don't release many interferons — these are signalling proteins that alert the immune system to the presence of a virus.
- By the time the immune system does realize there is a virus, there is so much of it that immune-response proteins sometimes flood the bloodstream at a faster rate than normal — which can cause damage.

Renovation Station

- Once the virus has taken over host translation, it starts a home makeover, extensively remodelling the interior and exterior of the cell to its needs.
- First, some of the newly made viral spike proteins travel to the surface of the cell and poke out of the host-cell membrane.
- At this point, the infected cell fuses to neighbouring cells expressing ACE2, developing into massive individual respiratory cells filled with up to 20 nuclei. These mega structures are called syncytia.
- Some COVID-19-infected cells even form syncytia with lymphocytes — one of the body's own immune cells. This is a known mechanism of immune evasion by tumour cells, but not by viruses. It suggests that infected cells avoid immune detection by simply grabbing on to and merging with nearby immune scouts.
- Like other coronaviruses, SARS-CoV-2 transforms the long, thin endoplasmic reticulum (ER), a network of flat membranes involved in protein synthesis and transport, into double-membrane spheres.
- Proteins involved in making DMVs could be good drug targets, because they seem to be necessary for viral replication. For instance, a host protein,

TMEM41B, is needed to mobilize cholesterol and other lipids to expand the ER membranes so that all the virus parts will fit inside.

- The coronavirus transmembrane protein Nsp3 could also be a target: it creates a crown-like pore in the walls of the DMVs to shuttle out newly made viral RNA.
- Usually, virus particles exit the cell using Golgi apparatus but SARS-CoV-2 also uses lysosomes. This is novel behaviour. The researchers are currently testing inhibitors that block the lysosomal exit process as potential antiviral candidates.

Last Slice

- On the way out of the cell, one more event makes this virus into an infectious juggernaut: a quick snip at a site of five amino acids prepares the virus to strike its next target.
- Furin is suspected to cut the site at some point during virion assembly, or just before release. The timing might explain why the virus exits through the Golgi or lysosomes
- Coronavirus with an intact furin cleavage site enters human airway cells faster than do those without it.
- Two coronavirus variants, Alpha and Delta, have altered furin cleavage sites. In the Alpha variant, the initial proline amino acid is changed to a histidine (P681H) ; in the Delta variant, it is changed to an arginine (P681R). Both changes make the sequence less acidic, and the more basic the string of amino acids, the more effectively furin recognizes and cuts it.
- We would hypothesize that this is the virus getting even better at transmitting.
- More furin cuts mean more spike proteins primed to enter human cells. In SARS-CoV, less than 10% of spike proteins are primed.
- In SARS-CoV-2, that percentage rises to 50%. In the Alpha variant, it’s more than 50%. In the highly transmissible Delta variant, the group has found, greater than 75% of spikes are primed to infect a human cell.

Known Unknowns

- The number of ACE2 receptors needed to bind to each spike protein
- When exactly the S2 site is cleaved by TMPRSS2
- And the number of spikes needed for virus–cell membrane fusion
- Most mutations so far are associated with how effectively the virus spreads, not with how much the virus damages the host.